JOURNAL OF LABELLED COMPOUNDS AND RADIOPHARMACEUTICALS
*J Label Compd Radiopharm* 2005; **48**: 721–733.
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jlcr.965

**Research Article**

# Synthesis of (*R*)- and (*S*)-[*O-methyl*-$^{11}$C]*N*-[2-[3-(2-cyano-phenoxy)-2-hydroxy-propylamino]-ethyl]-*N*′-(4-methoxy-phenyl)-urea as candidate high affinity $\beta_1$-adrenoceptor PET radioligands

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## Summary

Molecular imaging and quantification of myocardial $\beta_1$-adrenoceptor (AR) rather than total $\beta$-AR density is of great clinical interest since cardiac biopsy studies suggest that myocardial $\beta_1$-AR density is reduced in patients with chronic heart failure whereas cardiac $\beta_2$-AR density may vary. Positron emission tomography (PET), with appropriate radioligands, offers the possibility to assess $\beta$-AR density non-invasively in humans. However, no PET radioligand for the selective imaging of cardiac $\beta_1$-ARs is clinically available. Here some derivatives of the well characterized $\beta_1$-AR selective antagonist, ICI 89,406, namely the enantiomers of *N*-[2-[3-(2-cyano-phenoxy)-2-hydroxy-propylamino]-ethyl]-*N*′-(4-hydroxy-phenyl)-urea (**5a** and **5b**) were synthesized and evaluated *in vitro*. The (*R*)-isomer **5a** was more $\beta_1$-selective but has lower affinity than its (*S*)-enantiomer **5b** ($\beta_1$-AR selectivity: 6100 vs 1240; $\beta_1$-affinity: $K_1 = 0.288$ nM vs $K_1 = 0.067$ nM). Etherification of the analogous desmethyl precursors, **5e** and **5f**, respectively, with [$^{11}$C]iodomethane gave $^{11}$C-labelled versions of **5a** and **5b**, namely **5g** and **5h**, in 44 ± 5% radiochemical yield (decay-corrected) and 97.4 ± 1.3% radiochemical purity with specific radioactivities of 26.4 ± 9.4 GBq/μmol within 41.2 ± 3.4 min from the end of bombardment ($n = 14$). **5g** and **5h** are now

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Contract/grant sponsor: DFG; contract/grant number: SFB 556
Contract/grant sponsor: DFG; contract/grant number: SCHA 758/5-1
Contract/grant sponsor: SFB; contract/grant number: 656



*Received 24 February 2005*
*Revised 12 April 2005*
*Accepted 2 May 2005*

being evaluated as candidate radioligands for myocardial $\beta_1$-ARs. 

**Key Words:** 3-aryloxy-2-propanolamine; ICI 89,406; $\beta_1$-adrenoceptor selective ligand; PET radioligand

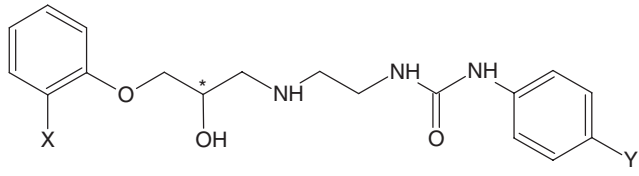
## Introduction

$\beta$-ARs belong to the rhodopsin/$\beta_2$-adrenergic receptor-like receptors, that represent a subfamily of the G protein-coupled-receptors (GPCRs).[1] They can be subdivided into at least three discrete subtypes, the $\beta_1$-, $\beta_2$-AR[2] and the atypical $\beta_3$-AR.[3,4] $\beta_1$-AR agonists cause increased cardiac contractility and heart rate while $\beta_2$-selective AR agonists mediate vasodepression and bronchodilation.[5] The so-called atypical $\beta_3$-ARs are involved in lipolysis.[3]

In heart disease, both the total $\beta$-AR density and the $\beta_1/\beta_2$-AR ratio may change. For example, reduced myocardial $\beta$-AR density has been found in hypertension, heart failure, ischaemia and both hypertrophic (HCM) and dilated cardiomyopathies (DCM).[6–11]

A selective reduction in $\beta_1$-ARs without change in $\beta_2$-AR density is often observed in the failing human heart.[5] Therefore, a means for the visualization and quantification of the $\beta_1$-AR density rather than total $\beta$-AR density in the human heart is of great interest in basic research and clinical application.[12] The molecular imaging techniques, single photon emission computed tomography (SPECT) and positron emission tomography (PET), with suitable radioligands may be ideal tools for assessing $\beta$-AR density non-invasively in humans.[13,14] Some subtype-$\beta_1$-AR selective radioligands have been developed for PET, for example (+/−)-[$^{11}$C]HX-CH 44,[15] (*S*)-[$^{11}$C]bisoprolol,[16] [$^{11}$C]CGP 20712A[17] and its (*S*)-enantiomer, [$^{11}$C]CGP-26505.[18] The clinical use of these radioligands, however, is limited because of high non-specific binding, rapid metabolism or tissue uptake that does not reflect binding to $\beta$-ARs.[15–18]

ICI 89,406 (Scheme 1) is an example of a designed $\beta_1$-selective AR antagonist. Although membrane studies show that the (*S*)-enantiomer is the more potent enantiomer even the racemate produces effective $\beta_1$-AR blockade during exercise in patients with angina pectoris.[19–21] Therefore, we chose this compound as a lead structure to develop new $\beta_1$-selective AR radioligands. In our initial study, the $\beta_1$-AR affinity and selectivity of a number of novel derivatives of ICI 89,406 were determined.[22] One of these derivatives, racemic I-ICI-H and its radioiodinated analogue (Scheme 1), showed high affinity and selectivity for $\beta_1$-ARs in myocardial membranes *in vitro* but high non-specific binding *in vivo*.[23] Another derivative, racemic I-ICI-COOH and its (*S*)-enantiomer, were also characterized by high $\beta_1$-AR affinity and selectivity *in vitro*.[24] The $^{125/123}$I-labelled counterparts (Scheme 1) were evaluated as




| | | | |
|---|---|---|---|
| ICI 89,406 | X: CN | Y: H | racemic |
| I-ICI-H | X: I, $^{125}$I or $^{123}$I | Y: H | racemic |
| I-ICI-COOH | X: I, $^{125}$I or $^{123}$I | Y: COOH | (*S*) or racemic |

**Scheme 1. Lead structure ICI 89,406 and its iodinated derivatives[23,24]**

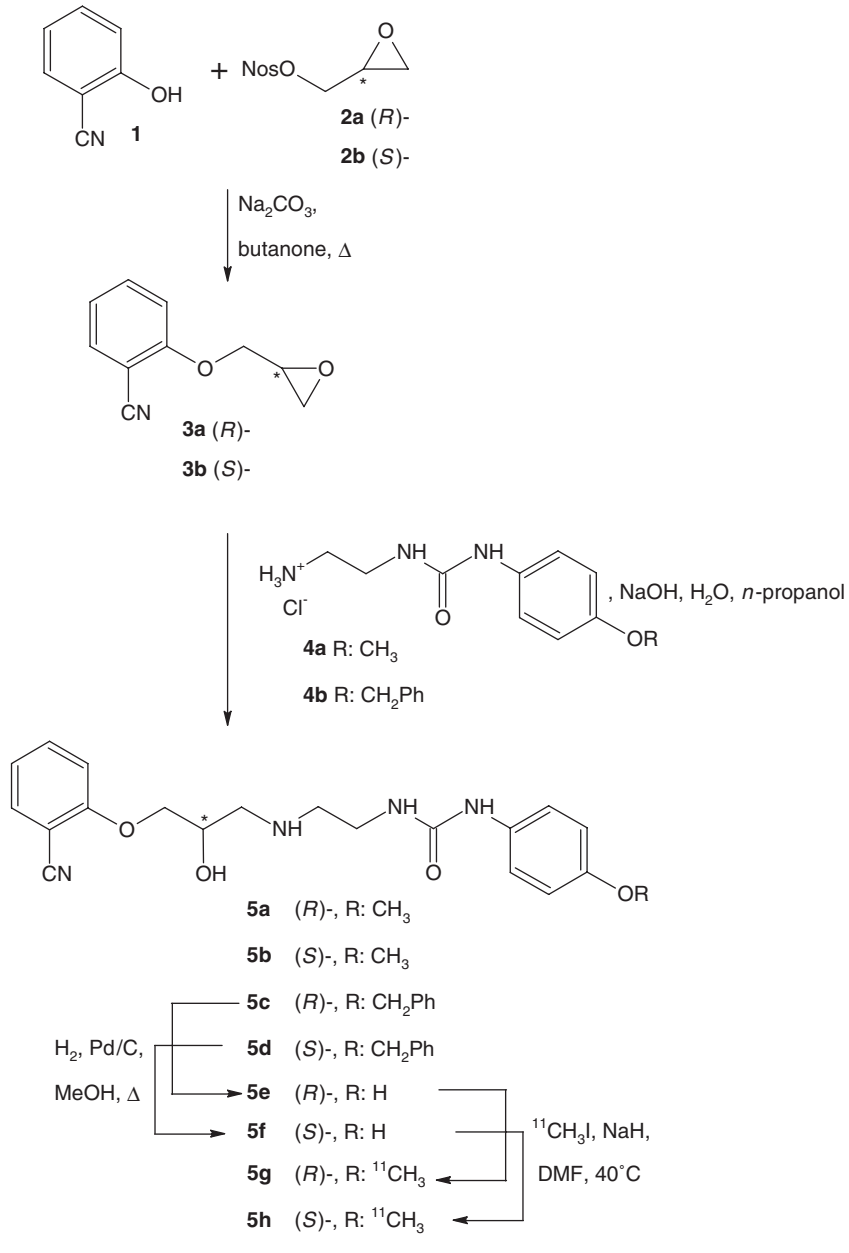
potential SPECT ligands using Sprague Dawley rats but were unsuitable because of a high degree of deiodination.[24] It is apparent that high non-specific binding and rapid deiodination *in vivo* preclude the use of such iodinated ligands for SPECT or PET. Ligands labelled with the positron-emitter, carbon-11 ($t_{1/2} = 20.4$ min), may be less susceptible to problematic metabolism. The work presented here describes the synthesis and the *in vitro* pharmacology of the non-iodinated ICI 89,406 derivatives, (*R*)- and (*S*)-*N*-[2-[3-(2-cyano-phenoxy)-2-hydroxy-propylamino]-ethyl]-*N*′-(4-methoxy-phenyl)-urea (**5a** and **5b**, respectively) and their precursors (**5c**–**5f**). The *in vitro* studies showed that the (*R*)-isomer (**5a**) is more selective but has lower affinity for $\beta_1$-ARs than the (*S*)-isomer (**5b**). Therefore, $^{11}$C-labelled versions of each enantiomer (**5g** and **5h**) were synthesized as potential selective and high affinity $\beta_1$-AR radioligands for PET (Scheme 2).

## Results and discussion

The syntheses of the ureas **5a**–**f** were achieved through a five- or six-step sequence (Scheme 2). Treatment of the homochiral glycidyl-3-nitrobenzene sulphonates **2a** and **2b** with 2-cyano-phenol **1** under basic conditions yielded the corresponding oxiranes **3a** and **3b** in good yields (85–92%). Comparison of the measured optical rotations with literature values showed that the stereochemistry was retained; the absolute $[\alpha]_D$ values were within errors.[25] The ureas **5a**–**d** were prepared from the oxiranes **3a** and **3b** and the amines **4a** and **4b** via nucleophilic ring opening. Compounds **4a** and **4b** were synthesized through a three-step sequence as previously described (steps not shown in Scheme 2).[22] The chemical yields ranged from 35 to 45%. Finally, **5c** and **5d** were deprotected by hydrogenolysis resulting in the phenol precursors **5e** and **5f** (51–56%).

The structure–activity relationships (SAR) between the ligands and $\beta_1$-ARs were assessed in competition studies using [$^{125}$I]ICYP and mouse ventricular




**Scheme 2. Synthesis of the chiral $\beta_1$-AR ligands 5a–h**

membrane preparations. The high- and low-affinity $IC_{50}$ values for the $\beta_1$- and $\beta_2$-ARs of the non-radioactive 3-aryloxy-2-propanolamine derivatives **5a**–**f** were calculated by non-linear regression analysis of membrane radioactivity. The high- and low-affinity inhibition constants ($K_1$ for the $\beta_1$-ARs and $K_2$ for the $\beta_2$-ARs) were obtained from the $IC_{50}$ values by the method of

**Table 1. Inhibition constants and calculated $\beta_1$-AR selectivities of the ligands 5a–f determined by a radioligand binding assay using mouse ventricular membrane preparations, plus calculated ligand logP and logD values of the ligands**

| Compound | $K_1$ (nM)[a] | $K_2$ (nM)[a] | $\beta_1$-selectivity[b] | logP[c] | logD[c] |
|---|---|---|---|---|---|
| **5a** | 0.288 ± 0.032 | 1760 ± 300 | 6100 ± 1540 | 1.22 | −0.21 |
| **5b** | 0.067 ± 0.016 | 83 ± 33 | 1240 ± 280 | 1.22 | −0.21 |
| **5c** | 20.7 ± 4.2 | 3900 ± 150 | 188 ± 45 | 2.87 | 1.55 |
| **5d** | 0.085 ± 0.019 | 119 ± 19 | 1400 ± 160 | 2.87 | 1.55 |
| **5e** | 0.269 ± 0.067 | 569 ± 277 | 2120 ± 450 | 0.83 | −0.55 |
| **5f** | 0.328 ± 0.046 | 149 ± 27 | 453 ± 106 | 0.83 | −0.55 |
| ICI 89 406 | 0.28 ± 0.12 | 41 ± 3 | 149 ± 86 | 1.57 | 0.13 |

[a] Inhibition constants for non-selective $\beta$-AR ligand [$^{125}$I]ICYP binding at $\beta_1$- and $\beta_2$-ARs expressed in mouse ventricular membrane preparations given as mean ± SEM, $n = 3$.
[b] The ratios of the low- over the high-affinity inhibition constants ($K_2/K_1$) indicate the $\beta_1$-AR selectivities of the non-radioactive $\beta_1$-AR ligands, noted as mean ± SEM, $n = 3$.
[c] logP values of the neutral form and logD values calculated by ACD/LogD Suite (logD = logP at physiological pH 7.4 with consideration of charged species).

Cheng–Prusoff[26] using the previously experimentally determined $K_D$ value of [$^{125}$I]ICYP (32.3 ± 1.9 pM).[22] The ratios of the low- to high-affinity inhibition constants ($K_2/K_1$) yield the $\beta_1$-selectivities of the unlabelled compounds **5a–f** (Table 1). The calculated logP and logD values (ACD/LogD Suite) of compounds **5a–f** are also listed in Table 1 and indicate the change in lipophilicities ensuing chemical modifications of the lead compound, ICI 89,406. The logP and logD values of **5a** and **5b** are low and similar to the values for (*S*)-CGP 12177 (logP = 1.81),[13] which when labelled with carbon-11 is an effective radioligand for imaging cell surface $\beta$-ARs in human myocardium with PET. All compounds **5a–f** possess high affinity to $\beta_1$-ARs. With the exception of **5c** ($K_1 = 20.7$ nM) the ligands showed $\beta_1$-AR affinities in the subnanomolar range ($K_1 = 0.067$–$0.328$ nM).

Usually the (*S*)-enantiomer of a $\beta$-blocker possesses an up to 100-fold higher affinity to $\beta$-ARs than the corresponding (*R*)-enantiomer.[27,28] This feature was shown by the ligands **5a** and **5b**. The (*R*)-enantiomer **5a** has at least four times less $\beta_1$-AR affinity than the (*S*)-isomer **5b** ($K_1 = 0.288$ nM vs $K_1 = 0.067$ nM). However, the $\beta_1$-AR selectivity of **5a**, is five times greater than that of **5b** (6100 vs 1240). Furthermore, the $\beta_1$-AR selectivities of the methoxy compounds **5a** and **5b** are at least an order of magnitude greater than that of the racemic lead compound, ICI 89,406 (149). A similar trend is seen in the benzyloxy-pair **5c** and **5d**. The (*R*)-enantiomer **5c** has about 240-times less $\beta_1$-affinity than the (*S*)-isomer **5d** ($K_1 = 20.7$ nM vs $K_1 = 0.085$ nM). In contrast, the (*S*)-enantiomer **5d** exhibits higher $\beta_1$-AR selectivity than its (*R*)-enantiomer **5c** (1400 vs 188). Contrary to expectations, both enantiomers of the hydroxy-pair **5e** and **5f** display nearly equal $\beta_1$-AR affinities ($K_1 = 0.269$ and $0.328$ nM). Similar to

compounds **5a** and **5b**, the (*R*)-enantiomer **5e** possesses a nearly five-fold higher $\beta_1$-AR selectivity than its (*S*)-enantiomer **5f** (2120 vs 453).

For the compounds **5a**–**f**, with their specific substitution pattern of a 2-cyanophenyloxy core and a 4-hydroxyphenyl or an 4-alkoxyphenyl unit, two SAR-conclusions can be drawn. Firstly, a more sterically demanding residue and/or a more lipophilic aromatic substituent at the oxygen of the phenyl-4-position (**5c** and **5d** R:Bn) leads to an increased difference between the $\beta_1$-AR affinities of the (*R*)- and (*S*)-enantiomers compared to enantiomer-pairs with a smaller substituent at this position (**5a** and **5b** R:Me; **5e** and **5f** R:H). The (*R*)-enantiomer **5c** has 244-fold less $\beta_1$-affinity than the (*S*)-enantiomer **5d**. The tendency is reduced in the methoxy-pair **5a** and **5b** (four-fold) and the hydroxy-pair **5e** and **5f** (0.8-fold). Secondly, an (*S*)-enantiomer with a benzyl-substituent at the phenolic oxygen is more $\beta_1$-selective than its (*R*)-enantiomer, whereas smaller substituents shift the increased $\beta_1$-AR selectivity to the (*R*)-enantiomer ((*R*)-enantiomer to (*S*)-enantiomer ratios of $\beta_1$-AR selectivities: **5c** and **5d** R:Bn: 0.13; **5a** and **5b** R:Me: 4.9; **5e** and **5f** R:H:4.7).

As a result of these binding affinity determinations, the potent methoxy derivatives **5a** and **5b** were chosen for conversion into corresponding potential high affinity and selective $\beta_1$-AR radioligands for PET, namely **5g** and **5h**, respectively. Thus, the desmethyl precursors **5e** and **5f** were treated with sodium hydride and [$^{11}$C]iodomethane in DMF at 40°C for 5 min to yield $^{11}$C-labelled **5g** and **5h**, respectively (Scheme 2), which were then purified with HPLC. The activities of **5g** and **5h** ranged from 1.2 to 2.7 GBq (EOS), representing $44.1 \pm 5.0\%$ decay-corrected radiochemical yields at $41.2 \pm 3.4$ min from the end of radionuclide production. Radiochemical purities of $97.4 \pm 1.3\%$ and specific activities of $26.4 \pm 9.4$ GBq/μmol (13.3–47.4 GBq/μmol) were achieved ($n = 14$), as determined by analytical radio-HPLC. The new radioligands **5g** and **5h** are now being evaluated in rodents with PET.

## Experimental

### *General methods*

All chemicals, reagents, and solvents for the synthesis of the compounds were analytical grade and purchased from commercial sources.

The melting points (uncorrected) were determined on a Stuart Scientific SMP3 capillary melting point apparatus. $^1$H-NMR and $^{13}$C-NMR spectra were recorded on a Bruker ARX 300 spectrometer and an AMX 400 spectrometer, respectively. Mass spectrometry was performed using a Varian MAT 212 (EI = 70 eV) spectrometer and a Bruker MALDI-TOF-MS Reflex IV (matrix: DHB). $[\alpha]_D$ values were determined on a Perkin-Elmer 341 polarimeter. Elemental analysis was realised by a Vario EL III analyser.

Radiosyntheses were carried out using an automated PET tracer synthesizer (TRACERLab $Fx_C$; GE Functional Imaging GmbH). Separation of the radiosynthesized compounds, and determinations of the radiochemical yields were performed with radio-HPLC using a Syknm S1021 pump, a Knauer K-2001 UV-detector (wavelength 254 nm), a Raytest Ramona-90/92 $\gamma$-detector, a Nucleosil 100-10 C18 precolumn ($20 \times 8\,mm^2$) and a Nucleosil 100-10 C18 column ($250 \times 8\,mm^2$). Sample injection was carried out using a VICI injector block (type C6W incl. 1000 µl loop). The recorded data were processed by the TRACERLab C software (GE Functional Imaging GmbH). The radiochemical purities and the specific activities were acquired with a radio-HPLC system composed of a Syknm S1021 pump, a Knauer K-2501 UV-detector (wavelength 254 nm), a Crismatec Na(Tl) Scintibloc 51 SP51 $\gamma$-detector, a Nucleosil 100-3 C18 column ($200 \times 3\,mm^2$), a VICI injector block (type C1 incl. 20 µl loop) and the NINA version 4.8, Rev. 4 software (GE Functional Imaging GmbH).

*Synthetic methods*

*Synthesis of oxiranes (***3a–b***)*

*General procedure*. 2-Cyano-phenol **1** (2 eq.), (*R*)-glycidyl-3-nitrobenzene sulphonate **2a** or (*S*)-glycidyl-3-nitrobenzene sulphonate **2b** (1 eq.) and anhydrous $K_2CO_3$ (6 eq.) were refluxed in dry 2-butanone (3.8 ml/mmol sulphonate) for 6–7 h and then stirred at RT for 16–72 h under an argon atmosphere. The mixture was filtered and the filter cake washed with 2-butanone. Solvent from the combined filtrates was evaporated off. The residue was dissolved in water and $CH_2Cl_2$. The pH of the aqueous layer was adjusted to be within the range 11–13 and the aqueous layer was extracted. The layers were separated. The aqueous layer was extracted with $CH_2Cl_2$ twice, the combined organic layers dried ($Na_2SO_4$) and the solvent evaporated off. The residue was recrystallized from diisopropyl ether–$CHCl_3$ ($\sim$3:2 v/v) at $-30$°C to provide **3a**–**b** as colourless solids.

*(R)-2-(2-cyano-phenoxymethyl)-oxirane (***3a***)*. Yield: 85%. Mp.: 85–86°C. $^1$H-NMR (300 MHz, $CDCl_3$): δ [ppm]: 7.58–7.50 (m, 2H, $H_{Ar}$), 7.09–7.01 (m, 2H, $H_{Ar}$), 4.37 (dd, $^2J = 11.6$ Hz, $^3J = 3.2$ Hz, 1H, $1CH_2$), 4.12 (dd, $^2J = 11.6$ Hz, $^3J = 5.3$ Hz, 1H, $1CH_2$), 3.40–3.37 (m, 1H, CH), 2.93 (dd, $^2J = 4.8$ Hz, $^3J = 4.2$ Hz, 1H, $1CH_2$), 2.84 (dd, $^2J = 5.0$ Hz, $^3J = 2.4$ Hz, 1H, $1CH_2$). $^{13}$C-NMR (75.5 MHz, $CDCl_3$): δ [ppm]: 160.01, 134.35, 133.87, 121.44, 116.26, 112.79, 102.50, 69.49, 49.85, 44.55. $[\alpha]_D^{20} = -15.8°$ ($c = 1.0$, EtOH); Lit.: $[\alpha]_D^{25} = -16.0°$ ($c = 1.0$, EtOH).[25] Analytically calculated for $C_{10}H_9NO_2$: C 68.56, H 5.12, N 8.00. Found: C 68.93, H 5.07, N 8.16.

*(S)-2-(2-cyano-phenoxymethyl)-oxirane (***3b***)*. Yield: 92%. Mp.: 86°C; Lit.: 88–89°C.[25] $^1$H-NMR (300 MHz, $CDCl_3$): δ [ppm]: 7.58–7.49 (m, 2H, $H_{Ar}$), 7.10–7.01 (m, 2H, $H_{Ar}$), 4.37 (dd, $^2J = 11.4$ Hz, $^3J = 3.0$ Hz, 1H, $1CH_2$), 4.12 (dd, $^2J = 11.4$ Hz, $^3J = 5.4$ Hz, 1H, $1CH_2$), 3.41–3.37 (m, 1H, CH), 2.93 ('t', $J = 4.5$ Hz, 1H, $1CH_2$), 2.84 (dd, $^2J = 5.0$ Hz, $^3J = 2.6$ Hz, 1H, $1CH_2$). $^{13}$C-NMR (75.5 MHz, CDCl3): δ [ppm]: 159.99, 134.35, 133.84, 121.42, 116.26, 112.80, 102.45, 69.47, 49.85, 44.54. $[\alpha]_D^{20} = +18.1°$ ($c = 1.0$, EtOH); Lit.: $[\alpha]_D^{25} = +17.69°$ ($c = 1.0$, EtOH).[25] Analytically calculated for $C_{10}H_9NO_2$: C 68.56, H 5.12, N 8.00. Found: C 68.80, H 4.96, N 8.38.

*Synthesis of the N-aryl-N′-[2-[3-aryloxy-2-hydroxy-propylamino]-ethyl]-urea derivatives (***5a–d***)*

*General procedure*. **3a** or **3b** (1 eq.), *N*-(2-amino-ethyl)-*N′*-(4-methoxy-phenyl)-urea hydrochloride **4a** (1 eq.) or *N*-(2-amino-ethyl)-*N′*-(4-benzyloxy-phenyl)-urea hydrochloride **4b** (1 eq.), prepared as previously described,[22] and 1 N NaOH (1.05 eq.) were heated in *n*-propanol (1.8–2.9 ml/mmol oxirane) and water (1.0–2.4 ml/mmol oxirane) up to 90°C for 2–3 h.

Purification procedure A (**5a** and **5c**): The mixture was evaporated to dryness. Water was added and stirred. The product was filtered off and dried *in vacuo*. The crude product was refluxed in methanol, the hot suspension was filtered and the filtrate was left for crystallization at +4 to −30°C. Additional amounts of product were obtained by crystallization from the mother liquor.

Purification procedure B (**5b** and **5d**): Water was added. The product was filtered off and washed with water–diethyl ether. The crude product was purified by silica gel chromatography (ethyl acetate–MeOH 4:1 v/v). The product fraction was evaporated and recrystallized from methanol.

After the purification procedures A or B, the urea derivatives **5a–d** were obtained as colourless solids.

*(R)-N-[2-[3-(2-cyano-phenoxy)-2-hydroxy-propylamino]-ethyl]-N′-(4-methoxy-phenyl)-urea (***5a***)*. Yield: 35%. Mp.: 126–127°C. $^1$H-NMR (400 MHz, DMSO-$d_6$): δ [ppm]: 8.29 (s, 1H, NH), 7.69 (dd, $^3J = 7.7$ Hz, $^4J = 1.7$ Hz, 1H, $H_{Ar}$), 7.61 (ddd, $^3J = 8.7$ Hz, $^3J = 7.4$ Hz, $^4J = 1.4$ Hz, 1H, $H_{Ar}$), 7.27–7.23 (m, 3H, $H_{Ar}$), 7.06 (dt, $^3J = 7.5$ Hz, $^4J = 0.9$ Hz, 1H, $H_{Ar}$), 6.78 (dd, $^3J = 6.8$ Hz, $^4J = 2.3$ Hz, 2H, $H_{Ar}$), 6.02 (t, $^3J = 5.1$ Hz, 1H, NH), 5.01 (s, br, 1H, OH), 4.16–3.92 (m, 3H, $CH_2CH$), 3.67 (s, 3H, $CH_3$), 3.14 (q, $^3J = 5.9$ Hz, 2H, $CH_2$), 2.75–2.60 (m, 4H, $2CH_2$). $^{13}$C-NMR (100.6 MHz, DMSO-$d_6$): δ [ppm]: 160.52, 155.71, 154.03, 135.14, 133.89, 133.76, 121.13, 119.53, 116.58, 114.02, 113.35, 100.83, 71.79, 68.12, 55.30, 52.13, 49.53, 39.89. Analytically calculated for $C_{20}H_{24}N_4O_4$: C 62.49, H 6.29, N 14.57. Found: C 62.25, H 6.24, N 14.62.

*(S)-N-[2-[3-(2-cyano-phenoxy)-2-hydroxy-propylamino]-ethyl]-N′-(4-methoxy-phenyl)-urea (***5b***)*. Yield: 45%. Mp.: 128–129°C. $^1$H-NMR (400 MHz, DMSO-$d_6$): δ [ppm]: 8.30 (s, 1H, NH), 7.69 (d, $^3J$ = 7.8 Hz, 1H, $H_{Ar}$), 7.62 ('t', $^3J$ = 7.4 Hz, 1H, $H_{Ar}$), 7.27–7.23 (m, 3H, $H_{Ar}$), 7.06 (t, $^3J$ = 7.5 Hz, 1H, $H_{Ar}$), 6.78 (d, $^3J$ = 8.7 Hz, 2H, $H_{Ar}$), 6.03 (t, $^3J$ = 5.3 Hz, 1H, NH), 5.02 (s, br, 1H, OH), 4.16–3.90 (m, 3H, $CH_2CH$), 3.68 (s, 3H, $CH_3$), 3.15 (q, $^3J$ = 5.7 Hz, 2H, $CH_2$), 2.76–2.61 (m, 4H, $2CH_2$). $^{13}$C-NMR (100.6 MHz, DMSO-$d_6$): δ [ppm]: 160.50, 155.68, 154.01, 135.13, 133.89, 133.77, 121.13, 119.48, 116.54, 114.01, 113.35, 100.82, 71.76, 68.05, 55.29, 52.09, 49.49, 39.91. Analytically calculated for $C_{20}H_{24}N_4O_4$: C 62.49, H 6.29, N 14.57. Found: C 62.11, H 6.12, N 14.17.

*(R)-N-(4-benzyloxy-phenyl)-N′-[2-[3-(2-cyano-phenoxy)-2-hydroxy-propylamino]-ethyl]-urea (***5c***)*. Yield: 41%. Mp.: 117–118°C. $^1$H-NMR (300 MHz, DMSO-$d_6$): δ [ppm]: 8.31 (s, 1H, NH), 7.68 (d, $^3J$ = 7.5 Hz, 1H, $H_{Ar}$), 7.63–7.58 (m, 1H, $H_{Ar}$), 7.43–7.23 (m, 8 H, $H_{Ar}$), 7.06 (t, $^3J$ = 7.4 Hz, 1H, $H_{Ar}$), 6.87 (d, $^3J$ = 9.0 Hz, 2H, $H_{Ar}$), 6.04 (t, $^3J$ = 5.6 Hz, 1H, NH), 5.02 (s, 3H, OH and $CH_2$), 4.16–3.90 (m, 3H, $CH_2CH$), 3.15 (q, $^3J$ = 5.1 Hz, 2H, $CH_2$), 2.76–2.58 (m, 4H, $2CH_2$), 1.85 (s, br, 1H, NH). $^{13}$C-NMR (75.5 MHz, DMSO-$d_6$): δ [ppm]: 160.48, 155.63, 153.03, 137.52, 135.08, 134.13, 133.74, 128.46, 127.78, 127.69, 121.08, 119.39, 116.54, 115.06, 113.32, 100.81, 71.74, 69.59, 68.08, 52.12, 49.49. Analytically calculated for $C_{26}H_{28}N_4O_4$: C 67.81, H 6.13, N 12.17. Found: C 67.56, H 6.04, N 12.11.

*(S)-N-(4-benzyloxy-phenyl)-N′-[2-[3-(2-cyano-phenoxy)-2-hydroxy-propylamino]-ethyl]-urea (***5d***)*. Yield: 36%. Mp.: 113–114°C. $^1$H-NMR (300 MHz, DMSO-$d_6$): δ [ppm]: 8.31 (s, 1H, NH), 7.68 (d, $^3J$ = 7.5 Hz, 1H, $H_{Ar}$), 7.63–7.58 (m, 1H, $H_{Ar}$), 7.43–7.23 (m, 8 H, $H_{Ar}$), 7.06 (t, $^3J$ = 7.5 Hz, 1H, $H_{Ar}$), 6.87 (d, $^3J$ = 9.0 Hz, 2H, $H_{Ar}$), 6.04 (t, $^3J$ = 5.1 Hz, 1H, NH), 5.02 (s, 3H, OH and $CH_2$), 4.16–3.90 (m, 3H, $CH_2CH$), 3.15 (q, $^3J$ = 5.4 Hz, 2H, $CH_2$), 2.76–2.61 (m, 4H, $2CH_2$), 1.96 (s, br, 1H, NH). $^{13}$C-NMR (75.5 MHz, DMSO-$d_6$): δ [ppm]: 160.50, 155.64, 153.02, 137.53, 135.10, 134.15, 133.75, 128.45, 127.78, 127.70, 121.09, 119.40, 116.55, 115.08, 113.32, 100.81, 71.74, 69.59, 68.09, 52.12, 49.49, 39.84. Analytically calculated for $C_{26}H_{28}N_4O_4$: C 67.81, H 6.13, N 12.17. Found: C 62.48, H 5.97, N 12.16.

*Synthesis of the N-aryl-N′-[2-[3-aryloxy-2-hydroxy-propylamino]-ethyl]-urea derivatives (***5e–f***)*

*General procedure*. **5c** or **5d** (1 eq.), methanol (46 ml/mmol) and Pd/C (10%, 47 mg/mmol) were stirred in a $H_2$-atmosphere at RT for 15–16 h. Then the mixture was refluxed for 6–8.5 h. The hot reaction mixture was filtered and the

filtrate was evaporated to dryness. The residue was purified by silica gel chromatography (ethyl acetate–MeOH 4:1 v/v) to provide the urea derivative **5e** or **5f** ($R_f$: 0.15) as a colourless solid.

*(R)-N-[2-[3-(2-cyano-phenoxy)-2-hydroxy-propylamino]-ethyl]-N′-(4-hydroxy-phenyl)-urea (***5e***)*. Yield: 51%. Mp.: 120–122°C. $^1$H-NMR (300 MHz, DMSO-$d_6$): δ [ppm]: 8.87 (s, br, 1H, OH), 8.14 (s, 1H, NH), 7.69 (dd, $^3J = 7.7$ Hz, $^4J = 1.4$ Hz, 1H, $H_{Ar}$), 7.61 (ddd, $^3J = 8.4$ Hz, $^3J = 7.7$ Hz, $^4J = 1.1$ Hz, 1H, $H_{Ar}$), 7.24 (d, $^3J = 8.7$ Hz, 1H, $H_{Ar}$), 7.14–7.03 (m, 3H, $H_{Ar}$), 6.78 (dd, $^3J = 6.8$ Hz, $^4J = 2.1$ Hz, 2H, $H_{Ar}$), 5.98 (t, $^3J = 5.4$ Hz, 1H, NH), 4.98 (s, br, 1H, OH), 4.16–3.88 (m, 3H, $CH_2CH$), 3.13 (q, $^3J = 5.8$ Hz, 2H, $CH_2$), 2.75–2.59 (m, 4H, $2CH_2$). $^{13}$C-NMR (75.5 MHz, DMSO-$d_6$): δ [ppm]: 160.50, 155.77, 152.01, 135.11, 133.76, 132.27, 121.10, 119.93, 116.54, 115.19, 113.34, 100.81, 71.76, 68.07, 52.12, 49.55. Analytically calculated for $C_{19}H_{22}N_4O_4$: C 61.61, H 5.99, N 15.13. Found: C 61.24, H 5.98, N 14.92.

*(S)-N-[2-[3-(2-cyano-phenoxy)-2-hydroxy-propylamino]-ethyl]-N′-(4-hydroxy-phenyl)-urea (***5f***)*. Yield: 56%. Mp.: 118–119°C. $^1$H-NMR (300 MHz, DMSO-$d_6$): δ [ppm]: 8.87 (s, br, 1H, OH), 8.14 (s, 1H, NH), 7.69 (dd, $^3J = 7.7$ Hz, $^4J = 1.4$ Hz, 1H, $H_{Ar}$), 7.61 (ddd, $^3J = 8.7$ Hz, $^3J = 7.4$ Hz, $^4J = 1.5$ Hz, 1H, $H_{Ar}$), 7.24 (d, $^3J = 8.7$ Hz, 1H, $H_{Ar}$), 7.14–7.03 (m, 3H, $H_{Ar}$), 6.78 (dd, $^3J = 6.8$ Hz, $^4J = 2.0$ Hz, 2H, $H_{Ar}$), 5.97 (t, $^3J = 5.4$ Hz, 1H, NH), 5.01 (s, br, 1H, OH), 4.14–3.89 (m, 3H, $CH_2CH$), 3.13 (q, $^3J = 6.0$ Hz, 2H, $CH_2$), 2.75–2.60 (m, 4H, $2CH_2$). $^{13}$C-NMR (75.5 MHz, DMSO-$d_6$): δ [ppm]: 160.49, 155.81, 152.02, 135.12, 133.78, 132.27, 121.13, 119.96, 116.57, 115.18, 113.33, 100.82, 71.77, 68.08, 52.11, 49.56. Analytically calculated for $C_{19}H_{22}N_4O_4$: C 61.61, H 5.99, N 15.13. Found: C 61.14, H 5.94, N 14.88.

*Radiosynthesis of (R)- and (S)-[O-methyl-$^{11}$C]N-[2-[3-(2-cyano-phenoxy)-2-hydroxy-propylamino]-ethyl]-N′-(4-methoxy-phenyl)-urea (***5g** *and* **5h***)*. [$^{11}$C]Carbon dioxide was produced by the $^{14}N(p,\alpha)^{11}C$ nuclear reaction on research grade nitrogen containing 2.5% oxygen, using 11 MeV proton beams at currents of 40 μA from a CTI-RDS-111 cyclotron. The [$^{11}$C]carbon dioxide was trapped from the target gas in a stainless steel loop cooled with liquid nitrogen to −150°C. [$^{11}$C]Iodomethane was prepared from [$^{11}$C]carbon dioxide as previously described.[29] Phenol precursor **5e** or **5f** (1.0 mg; 2.70 μmol) was treated with NaH (60% oil suspension; 1.5 mg; 37.5 μmol) in DMF (200 μl) and [$^{11}$C]$CH_3I$ at 40°C for 5 min. After heating the reaction mixture to 50°C, water for injection (200 μl) was added and the crude mixture was loaded onto a semi-preparative HPLC-column (see Synthetic methods). Product **5g** or **5h** was eluted with 20 mM $Na_2HPO_4$-buffer/EtOH 4/1 at a flow of 4 ml/min at

11.9–14.5 min. The product solution was filtered through a sterile filter REF F60 (0.2 μm, pvb Medizintechnik GmbH). The time of synthesis and purification was 41.2 ± 3.4 min from the EOB. The radiochemical yield averaged 44.1 ± 5.0% (decay-corrected) and the radiochemical purity, determined via radio-HPLC (see Synthetic methods, eluent: $H_2O$/MeCN/TFA 700/300/1, flow: 0.3 ml/min, retention time: 11.1 min), was 97.4 ± 1.3% with a specific activity of 26.4 ± 9.4 GBq/μmol (13.3–47.4 GBq/μmol) at EOS ($n = 14$).

*Ligand binding assay*

Microsomes were prepared by homogenizing ventricles from DBA mice at 4°C for 90 s in buffer A (1 ml) containing 10 mM EDTA, 10 mM HEPES and 0.1 mM benzamidine (pH 7.4), using a Polytron PT 3000 (Kinematica, Lucerne, Switzerland). Homogenates were centrifuged at $45\,000 \times g_{max}$ for 15 min at 4°C. The pellets were resuspended again in buffer B (1 ml) containing 1 mM EDTA, 10 mM HEPES and 0.1 mM benzamidine (pH 7.4) and recentrifuged at $45\,000 \times g_{max}$ for 15 min at 4°C. The pellets were resuspended in buffer B (1 ml) and centrifuged at $10\,000 \times g_{max}$ for 10 min at 4°C. The supernatants were recentrifuged at $45\,000 \times g_{max}$ for 15 min at 4°C. The pellets, partially enriched membranes, were resuspended in buffer C (50 mM *Tris*·HCl, 5 mM $MgCl_2$ (pH 7.4)), and stored frozen at −80°C. For competition binding studies, the prepared membranes were resuspended in buffer D (10 mM *Tris*HCl, 154 mM NaCl, 0.1 mM ascorbic acid, pH 7.4). 15 μg of membranes were incubated with a constant concentration of [$^{125}$I]ICYP (80 pM) and with varying concentrations (1 pM–100 μM) of compounds **5a**–**f** (Scheme 2). Reactions were conducted at 37°C for 60 min. Reactions were stopped by filtering onto Whatman GF/B filters and washed with water for injection. The membrane bound radioactivity was determined in a $\gamma$-scintillation counter. Competition binding curves were analysed by non-linear regression analysis as previously described[30–32] using the XMGRACE program (Linux software). The data of the ligands **5a**–**f** fitted a two-site model significantly better than a one-site model ($F = 2.15$, $p < 0.05$). The resulting high- and low-affinity $IC_{50}$ values of all synthesized and non-radioactive 3-aryloxy-2-propanolamine derivatives **5a**–**f** were converted into the high- and low-affinity inhibition constants ($K_1$ for the $\beta_1$-ARs and $K_2$ for the $\beta_2$-ARs) by the method of Cheng–Prusoff[26] using the experimentally determined $K_D$ value of [$^{125}$I]ICYP (32.3 ± 1.9 pM).[22] The ratios of the low- to high-affinity inhibition constants ($K_2/K_1$) yield the $\beta_1$-selectivities of the unlabelled derivatives **5a**–**f** (Table 1). These compounds show a significantly higher affinity to $\beta_1$- than to $\beta_2$-ARs. Additionally, the calculated logP and logD values (ACD/LogD Suite) for compounds **5a**–**f** are listed in Table 1 to indicate

the changes of the lipophilicities caused by the chemical modifications of the lead compound, ICI 89,406.

## Conclusion

The syntheses, radiosyntheses and *in vitro* pharmacology of (*R*)- and (*S*)-*N*-[2-[3-(2-cyano-phenoxy)-2-hydroxy-propylamino]-ethyl]-*N*′-(4-methoxy-phenyl)-urea (**5a** and **5b**, respectively), and their $^{11}$C-labelled versions (**5g** and **5h**, respectively), potential new high affinity $\beta_1$-AR selective PET radioligands, are reported. The non-radioactive counterparts **5a** and **5b** of the radiolabelled target compounds **5g** and **5h** showed high affinity to $\beta_1$-ARs in murine myocardial membranes and moderate lipophilicity. The radiosyntheses of **5g** and **5h** were achieved with good radiochemical and acceptable specific activities. The formulation of the preparations is suitable for preliminary *in vivo* studies using small animal PET.

### Acknowledgements

This work was supported by the SFB 556, 656 and SCHA 758/5-1 of the Deutsche Forschungsgemeinschaft (DFG), Bonn, Germany. The authors gratefully thank Ms Monika Trub and Ms Sandra Schröer for technical assistance.

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